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CERTIFICATE OF MAILING

I hereby certify that this Application for Extension of Patent Term is being deposited with the U.S. Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington D.C. 20231, on June 28, 1995.

Pauline Ann Clarke — June 28, 1995
Pauline Ann Clarke, Reg. No. 29,783 — Date

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re : U.S. Patent No. 4,753,935

Issued: June 28, 1988

To: Syntex (U.S.A.) Inc., as assignee of Peter H. Nelson, Chee-Liang L. Gu, Anthony C. Allison, Elsie M. Eugui, and William A. Lee

Filed: January 30, 1987

App. No.: 07/008,717

For: MORPHOLINOETHYLESTERS OF MYCOPHENOLIC ACID AND PHARMACEUTICAL COMPOSITIONS

Honorable Commissioner of Patents and Trademarks
Box Pat. Ext.
Washington, D.C. 20231

Sir:

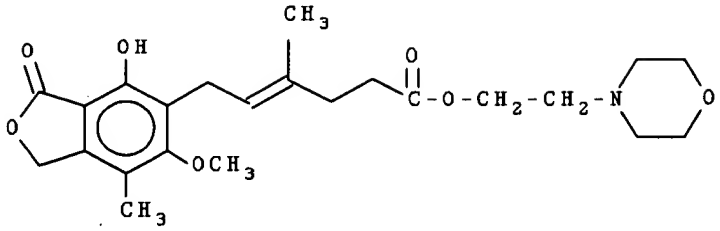
APPLICATION FOR EXTENSION OF PATENT TERM

Syntex (U.S.A.) Inc. submits this application for extension of the patent term of U.S. Patent No. 4,753,935 by providing the following information, as required by 35 U.S.C. 156 and 37 CFR 1.710 et seq.

(1) The approved product is CELLCEPT® (mycophenolate mofetil) 250 mg capsules.

It comprises a compound having:

(a) the following structural formula:



(b) the molecular formula: $C_{23}H_{31}NO_7$

(c) the molecular weight: 433.50

(d) the chemical names:

(1) (E)-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-isobenzofuranyl)-4-methyl-4-hexenoic acid, 2-(4-morpholinyl)ethyl ester; and

(2) 2-morpholinoethyl (E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoate

[as set forth in the 1995 *USAN and the USP Dictionary of Drug Names* at page 450, entry entitled **Mycophenolate Mofetil**];

(e) the generic name:

mycophenolate mofetil [USAN];

(f) the CAS registry number:

115007-34-6 .

(2) CELLCEPT® (mycophenolate mofetil) 250 mg capsules was subject to regulatory review under Section 507 of the Federal Food, Drug and Cosmetic Act (21 U.S.C. 357).

(3) CELLCEPT® (mycophenolate mofetil) 250 mg capsules received permission for commercial marketing under Section 507 of the Federal Food, Drug and Cosmetic Act (21 U.S.C. 357) on May 3, 1995.

(4) CELLCEPT® (mycophenolate mofetil) 250 mg capsules contain as the sole active ingredient mycophenolate mofetil, described

above in item (1). This product has not been previously approved for commercial marketing under Section 507 of the Federal Food, Drug and Cosmetic Act (21 U.S.C. 357); nor is this product covered by the claims of any patent which has been extended.

(5) This application for patent term extension is being submitted within the sixty day period permitted for submission under 37 CFR 1.720(f), the last day of which period is July 2, 1995.

(6) The patent for which patent term extension is sought is U.S. Patent No. 4,753,935, which issued on June 28, 1988 to Syntex (U.S.A.) Inc. as assignee of Peter H. Nelson, Chee-Liang L. Gu, Anthony C. Allison, Elsie M. Eugui, and William A. Lee on U.S. Patent Application No. 07/008,717, filed January 30, 1987, for MORPHOLINOETHYLESTERS OF MYCOPHENOLIC ACID AND PHARMACEUTICAL COMPOSITIONS. The term of U.S. Patent No. 4,753,935 will expire, unless extended, on January 30, 2007 [20 year patent term under 35 U.S.C. 154 (c)(1) as amended by the Uruguay Round Agreements Act (URAA), effective June 8, 1995; pre-URAA expiry date was June 28, 2005].

Syntex (U.S.A.) Inc. certifies that it is the assignee of the entire right, title, and interest in U.S. Patent No. 4,753,935 by virtue of an assignment from the inventors of U.S. Patent Application No. 07/008,717. The assignment was recorded in the U.S. Patent & Trademark Office on July 16, 1987 at Reel 4737, Frame 0220.

The undersigned has reviewed all documents in the chain of title of U.S. Patent No. 4,753,935 and, to the best of her knowledge and belief, title is in Syntex (U.S.A.) Inc. The undersigned is empowered to sign this certification on behalf of Syntex (U.S.A.) Inc.

(7) A copy of U.S. Patent No. 4, 753,935 is attached hereto as Attachment A.

(8) No disclaimer, certificate of correction, or reexamination certificate has issued in U.S. Patent No. 4,753,935. A receipt of maintenance fee payment issued in U.S. Patent No. 4,753,935 on November 14, 1991. A copy of this receipt is attached as Attachment B.

(9) U.S. Patent No. 4,753,935 claims CELLCEPT® (mycophenolate mofetil) 250 mg capsules in the following applicable claims:

Claim 1 covers *inter alia*, mycophenolate mofetil [a compound of the formula wherein Z is hydrogen];

Claim 2 covers mycophenolate mofetil;

Claim 12 covers *inter alia*, a pharmaceutical composition comprising a pharmaceutically acceptable non-toxic excipient and a therapeutically effective amount of mycophenolate mofetil [a compound of Claim 1 wherein Z is hydrogen].

(10) The relevant dates and information pursuant to 35 U.S.C. 156(g) and 37 CFR 1.740(10)(ii) are as follows:

June 24, 1988	Effective date of IND 31,747;
October 12, 1989	Effective date of IND 33,872;
November 10, 1994	Initial submission date of NDA 50-722 (originally submitted as NDA 20-513; changed by FDA to NDA 50-722 at approval);
May 3, 1995	Approval Date of NDA 50-722.

June 12, 1989	Syntex submits a revised draft protocol ("Nine Month Double Blind, Placebo-Controlled Multiple Dose Efficacy and Safety Study of RS-61443 in Patients with Rheumatoid Arthritis")
June 15, 1989	Syntex submits a new protocol for a study entitled, "Absorption of RS61443 When Administered with Food and Antacids."
June 27, 1989	Syntex submits a summary of study (see April 21, 1989 for title).
July 12, 1989	Syntex submits a revised draft protocol (see June 8, 1989 for title).
July 25, 1989	Syntex submits information on Chemistry, Manufacturing, and Controls. Contains stability statements for 50 mg, 100 mg & 250 mg capsules.
August 3, 1989	Syntex submits information amendment (Pharmacology/Toxicology reports).
August 8, 1989	Syntex submits information amendment (Pharmacology/Toxicology reports).
August 8, 1989	1st Annual Report (IND 31,747)
September 25, 1989	Syntex submits final protocol for study entitled, "One Year Open Label, Multiple Dose Efficacy and Safety Study of RS61443-000 in Patients with Rheumatoid Arthritis" (submitted in draft June 8, 1989), and also submits information amendment Pharmacology/Toxicology reports).
September 28, 1989	Syntex submits a revised protocol for a study entitled, "Randomized, Double Blind, Placebo Controlled Multiple Dose Safety and Dose Finding Study of RS61443-000 in Patients with Rheumatoid Arthritis."
October 12, 1989	Syntex submits an IND for immunosuppression

(11) A brief description of the activities undertaken by the applicant during the regulatory review period is as follows:

November 25, 1987	Syntex submits, at request of FDA, material for Pre-IND meeting.
January 6, 1988	Pre-IND meeting with FDA.
March 18, 1988	Syntex submits the minutes and copies of the transparencies from Pre-IND meeting.
June 14, 1988	Syntex receives FDA minutes from the Pre-IND meeting of January 6, 1988.
June 16, 1988	Syntex submits IND application to evaluate mycophenolate mofetil as a Disease Modifying Antirheumatic Drug (DMARD).
June 24, 1988	FDA sends Syntex acknowledgement of receipt (June 21, 1988) of submission with number assignment (IND 31,747).
November 1, 1988	Syntex submits information amendment (Pharmacology/Toxicology reports).
February 27, 1989	Syntex submits information amendment (Pharmacology/Toxicology reports).
March 9, 1989	Syntex receives letter from FDA re: chemistry/ pharmacology deficiencies.
March 27, 1989	Syntex submits information amendment (Pharmacology/Toxicology reports).
April 21, 1989	Syntex submits a revised protocol for a study entitled, "Randomized, Double Blind, Placebo Controlled Multiple Dose Safety and Dose Finding Study of RS61443-000 in Patients with Rheumatoid Arthritis."
June 8, 1989	Syntex submits a draft protocol for "Two Year Open-Label Multiple Dose Efficacy and Safety Study of RS-61443 in Patients with Rheumatoid Arthritis"

	following organ transplantation. (assigned IND 33,872).
October 16, 1989	Syntex submits additional Chemistry, Manufacturing and Controls information: Synthesis description for mycophenolate mofetil.
December 4, 1989	Syntex submits a draft protocol ("Nine-Month Double Blind, Placebo Controlled, Multiple Dose Efficacy and Safety Study in Patients with Rheumatoid Arthritis"). Syntex also submits pharmacokinetics and immunology summaries and the preliminary summary for the protocol submitted September 25, 1989.
December 21, 1989	Syntex responds to FDA's comments on IND 33,872.
January 10, 1990	Syntex submits information amendment (Pharmacology/Toxicology reports).
January 26, 1990	Syntex submits information amendment (Pharmacology/Toxicology reports).
February 9, 1990	Syntex submits information amendment (Pharmacology/Toxicology reports).
February 28, 1990	Syntex submits a new protocol for a study entitled "An Open-label, Multiple Dose, Safety and Dose Finding Study in Patients Following Renal Transplantation."
March 6, 1990	FDA informs Syntex that it is acceptable to proceed with proposed study (IND 33,872).
April 5, 1990	Syntex submits a final protocol for study (see June 12, 1989 entry); Syntex also submits a revised synthesis description and information amendment (Pharmacology/Toxicology reports).
April 11, 1990	Syntex submits a letter outlining two dose ranging safety and efficacy studies - one

	starting that week (see June 12, 1989 entry) and the second proposed to start in July.
May 7, 1990	Syntex submits additional manufacturing and control information for the placebo capsules.
July 9, 1990	Syntex submits for review, a draft protocol entitled "A Study of the Safety, Tolerance and Efficacy of 1 year of Therapy in Solid Organ Allograft Recipients."
July 23, 1990	Syntex submits a new protocol for a study entitled "An Open Label, Pilot, Dose Finding, Safety and Efficacy Study for Treatment of Cardiac Allograft Recipients."
July 31, 1990	Syntex submits a new protocol for a study entitled "A Study of the Safety, Tolerance and Efficacy of 1 Year of Therapy in Solid Organ Allograft Recipients."
August 14, 1990	Syntex submits information amendment (Pharmacology/Toxicology reports).
August 17, 1990	1st Annual Report (IND 33,872)
August 22, 1990	Syntex submits a protocol change extending study ICM 1733 from one year to two years.
September 13, 1990	2nd Annual Report (IND 31,747)
September 19, 1990	Syntex submits a new protocol for a study entitled, "Nine Month, Double Blind, Placebo Controlled, Multiple Dose Efficacy and Safety Study of RS61443-000 in Rheumatoid Arthritis Patients."
October 22, 1990	Syntex submits a letter to the FDA providing a statement of overall view of completed toxicology studies in the rat, monkey and dog and seeking agreement from the FDA that no further preclinical studies or clinical evaluations of the male gonadal function are

	warranted.
October 24, 1990	Syntex submits draft protocol for a study entitled "An Open Label, Pilot, Pharmacokinetic Safety and Efficacy Study for Treatment of Refractory Cellular Allograft Rejection."
November 27, 1990	Syntex submits information amendment (Pharmacology/Toxicology reports).
November 30, 1990	Syntex responds to March 9, 1989 FDA letter re chemistry/pharmacology deficiencies with a revised synthesis description and information on additional manufacturing sites.
December 3, 1990	Syntex submits a new protocol for a study entitled "An Open Label, Pilot, Pharmacokinetic Safety and Efficacy Study for Treatment of Refractory Cellular Allograft Rejection."
December 19, 1990	Syntex submits a new protocol for a study entitled, "One Year Open Label, Multiple Dose Efficacy and Safety Extension Study of RS61443-000 in Patients With Rheumatoid Arthritis."
January 16, 1991	Syntex submits draft protocols for rat and mouse carcinogenicity studies for review.
February 7, 1991	Syntex submits additional Chemistry, Manufacturing and Controls information for mycophenolate mofetil.
April 16, 1991	Syntex responds to March 27, 1991 discussion with FDA by submitting 2 final clinical study reports: (1) Double Blind Placebo Controlled Multiple Dose Safety and Dose Finding Study in Rheumatoid Arthritis Patients, and (2) Pharmacokinetic Interaction with Food or Antacids.
April 23, 1991	Syntex submits proposed procedures for handling IND safety reports.

May 1, 1991	Syntex receives facsimile of FDA letter with comments on draft carcinogenicity protocols.
May 6, 1991	Syntex submits information amendment (Pharmacology/Toxicology reports).
May 24, 1991	FDA submits comments on draft carcinogenicity protocols.
June 24, 1991	Syntex submits carcinogenicity protocols agreed upon between FDA and Syntex.
June 26, 1991	Syntex submits information on the effect of mycophenolate mofetil on interleukin pathways in response to FDA request.
June 27, 1991	Syntex submits a letter to the FDA agreeing to how safety reports for the two IND's will be reported. (result of telecon 6/14/91)
July 8, 1991	Syntex submits a revision of the mycophenolate mofetil synthesis.
August 7, 1991	Syntex submits information amendment (Pharmacology/Toxicology reports).
August 26, 1991	Syntex submits a Chemistry, Manufacturing and Controls amendment: stability statement for capsules.
September 3, 1991	Syntex submits information amendment (Pharmacology/Toxicology report).
September 26, 1991	Syntex submits revision of synthesis - describes alternate route.
September 30, 1991	Syntex submits safety and efficacy tables from interim analysis of the open label study (see June 12, 1989 for study title), and a draft protocol for a further clinical trial ("Nine Month Double Blind Multiple Dose Efficacy and Safety Comparison of Mycophenolate Mofetil 2000 mg Daily vs 3500 mg Daily in Rheumatoid Arthritis Patients").

September 30, 1991	3rd Annual Report (IND 31,747) and 2nd Annual Report (IND 33,872)
October 8, 1991	Syntex submits additional Chemistry, Manufacturing and Controls information: 250 mg capsule formulation; placebo capsules, and a stability statement for the 250 mg capsule formulation.
October 9, 1991	Syntex submits a new protocol for a study entitled "Two Year Open Label, Multiple Dose Efficacy and Safety Extension Study of Mycophenolate Mofetil in Patients with Rheumatoid Arthritis."
October 21, 1991	Syntex submits a letter to the FDA confirming agreement to do a single dose pharmacokinetic study in normal volunteers; also submits updated synthesis description.
November 4, 1991	Syntex submits stability information for formulations.
November 26, 1991	Syntex submits a new protocol for a study entitled "A Single Dose Bioavailability Study of Mycophenolate Mofetil 250 mg x 4 Capsules and Mycophenolate Mofetil 1000 mg Oral Solution."
December 23, 1991	Syntex submits a letter to the FDA with safety information in RA and transplant patients receiving at least 3 grams per day, and a brief synopsis of planned phase III RA studies.
January 21, 1992	Syntex submits draft protocol for "Open Label Study of Safety, Tolerance and Efficacy of 3 Years of Therapy with Mycophenolate Mofetil in Renal Transplant Patients."
March 17, 1992	Syntex submits a Chemistry, Manufacturing and Controls amendment to include an alternate

	finishing step in the recrystallization of mycophenolic acid.
April 1, 1992	Syntex submits final protocol for study (see September 30, 1991 entry).
April 20, 1992	Syntex submits information amendment (Pharmacology/Toxicology report).
April 29, 1992	Syntex submits revised method of manufacture data for formulations.
May 6, 1992	Syntex submits new protocol "Randomized Double Blind Comparative Study of Two Doses of Mycophenolate Mofetil or Azathioprine Each in Combination with Cyclosporine and Corticosteroids for Prevention of Rejection in Recipients of Their First Cadaveric Renal Allograft."
May 18, 1992	Syntex submits information amendment (Pharmacology/Toxicology reports).
June 9, 1992	Syntex met with FDA to discuss environmental fate and effect studies for mycophenolate mofetil.
June 12, 1992	FDA sends Syntex a letter pointing out two items that are relevant to the preclinical format of the summary to expedite the review process.
June 16, 1992	Syntex submits information amendment (Pharmacology/Toxicology report).
June 22, 1992	Syntex submits minutes of June 2, 1992 meeting with the FDA to discuss CMC issues for mycophenolate mofetil capsules.
July 1, 1992	Syntex submits an IND for the I.V. formulation.
July 10, 1992	Syntex submits dissolution data obtained of comparison of azathioprine capsules with commercially available azathioprine tablets.

July 15, 1992	FDA acknowledges the I.V. formulation IND application and assigns it number 40,050.
July 17, 1992	Syntex submits information amendment (Pharmacology/Toxicology reports).
August 4, 1992	Syntex submits additional dissolution data.
August 4, 1992	End-of-Phase II meeting held between FDA and Syntex to discuss clinical program for mycophenolate mofetil in kidney transplantation.
August 26, 1992	Syntex submits information amendment (Pharmacology/Toxicology reports).
September 4, 1992	Syntex submits minutes of August 4, 1992 meeting between FDA and Syntex.
September 8, 1992	3rd Annual Report (IND 33,872).
September 15, 1992	4th Annual Report (IND 31,747).
September 18, 1992	Syntex submits final minutes of a March 16, 1992 telephone conference with the FDA to discuss analyses from Phase II studies.
September 21, 1992	Syntex submits additional stability statements for five formulations.
September 30, 1992	FDA sends facsimile to Syntex on the FDA Pharmacologist's comments re: annual report.
October 8, 1992	Syntex receives letter from FDA forwarding comments on IND 40,050.
October 9, 1992	Syntex submits new protocol "An Open Label Safety, Dose Finding and Pharmacokinetic Pilot Study of Mycophenolate Mofetil in Combination with Cyclosporine and Corticosteroids in Cardiac Allograft Recipients Experiencing Acute Cellular Rejection."
October 15, 1992	Syntex receives October 1, 1992 letter from the FDA requesting that Syntex repeat the segment 1 fertility study in rats. Also requested that

	Syntex continue to discuss results of a preclinical trial in the investigators brochure.
October 16, 1992	Syntex submits a new protocol for a study entitled "Absorption, Metabolism and Excretion of a Single Oral Dose of ^{14}C Mycophenolate Mofetil Solution in Healthy Volunteers."
October 19, 1992	Syntex submits a new protocol, "A Single Dose Pharmacokinetic Study of Mycophenolate Mofetil in Subjects with Normal Renal Function and in Patients with Varying Degrees of Renal Function, Including Dialysis Patients."
October 22, 1992	Syntex submits new protocol, "An Open-Label, Multiple Dose, Safety, Dose Finding and Pharmacokinetic Pilot Study of Mycophenolate Mofetil in Combination with Steroids and Reduced Dose Cyclosporine in Patients Following Liver Transplantation."
November 19, 1992	Syntex submits a draft protocol for a study entitled "Placebo Controlled, Randomized, Double Blind Withdrawal from Mycophenolate Mofetil Followed by a 52-week, Open Label Extension Study in Rheumatoid Arthritis Patients."
December 21, 1992	Syntex submits a draft protocol for study "Bioequivalence of a Single Dose Mycophenolate Mofetil Given in Either 250 mg x 4 Capsules or 500 mg x 2 Tablets."
January 4, 1993	Syntex submits overview of toxicology studies completed and summaries of toxicology studies in rats and dogs in response to reviewer request.
February 17, 1993	Syntex submits a final protocol for a study

	(see December 21, 1992 entry).
February 19, 1993	Syntex submits background package with request for meeting to discuss clinical pharmacology/clinical pharmacokinetics program for mycophenolate mofetil.
February 25, 1993	Syntex submits minutes of June 9, 1992 meeting to discuss environmental fate and effect studies for mycophenolate mofetil, and Syntex requests a copy of FDA minutes of that meeting.
March 5, 1993	Syntex responds to FDA comments on IV formulation received October 8, 1992.
April 12, 1993	Syntex sends letter to FDA requesting a meeting to discuss chemistry, manufacturing and control for the IV dosage form.
April 14, 1993	Syntex submits information amendment (Pharmacology/Toxicology reports).
April 20, 1993	Syntex submits a draft protocol for a study entitled "Randomized, Controlled, Dose Ranging Study for the Safety, Pharmacokinetics, and Efficacy of Intravenous Followed by Oral Mycophenolate Mofetil in the Prevent of Acute Rejection in Primary Cadaveric Renal Allograft Recipients" for review and comments.
April 21, 1993	Meeting held between Syntex and FDA to discuss clinical pharmacology/clinical pharmacokinetics program for mycophenolate mofetil.
April 27, 1993	Syntex submits 2 draft protocols: (1) "A Single Dose Pharmacokinetic Drug Interaction Study of Oral Mycophenolate Mofetil and Oral Acyclovir in Normal Subjects" and (2) "Single Dose Pharmacokinetic Drug Interaction Study of Oral Mycophenolate Mofetil and IV Ganciclovir."
April 28, 1993	Syntex submits preliminary finished product

	release specifications at the request of the Division of Antiviral Drug Products, for oral capsules and tablets.
May 3, 1993	Syntex provides mortality rates and a summary of the gross necropsy finding from the ongoing carcinogenicity studies. Syntex requests a conference to discuss plans and reach agreement whether to continue the studies to the two year time line or to initiate terminal necropsy at the time a set number of animals is reached.
May 10, 1993	Syntex submits documentation of agreements reached with FDA regarding issues discussed on May 7, 1993 (subject of May 3, 1993 entry).
May 14, 1993	Syntex submits response to request for additional pk data from monkey study in support of study IID 2176.
May 17, 1993	Syntex submits draft minutes of April 21, 1993 meeting with FDA to discuss clinical pharmacology/clinical pharmacokinetics program for mycophenolate mofetil.
May 25, 1993	Syntex submits a new protocol for a study entitled, "An Open Label, Multiple Dose, Safety, Dose Finding and Pharmacokinetic Pilot Study of Mycophenolate Mofetil in Combination with Steroids and Reduced Dose Cyclosporine in Patients Following Liver Transplantation."
June 1, 1993	Syntex submits information amendment (Pharmacology/Toxicology reports).
June 2, 1993	Syntex receives comments on CMC issues regarding IV formulation from FDA
June 4, 1993	Syntex receives comments and recommendations from FDA regarding study IID 2176.
June 10, 1993	Syntex submits final protocol for study, "A

	Single Dose Pharmacokinetic Drug Interaction Study of Oral Mycophenolate Mofetil and Oral Acyclovir in Normal Subjects."
June 15, 1993	Syntex submits information amendment (Pharmacology/Toxicology reports).
June 16, 1993	Syntex submits final protocol for study, "Single Dose Pharmacokinetic Drug Interaction Study of Oral Mycophenolate Mofetil and IV Ganciclovir."
June 18, 1993	Syntex response to CMC comments received June 2, 1993. Syntex also submits new protocol for a study entitled "A Single Dose Pharmacokinetic Study of Mycophenolate Mofetil in Subjects with Normal Renal Function and in Patients with Varying Degrees of Renal Function, Including Dialysis Patients."
June 24, 1993	Syntex submits final minutes of April 21, 1993 meeting with FDA held to discuss clinical pharmacology/clinical pharmacokinetics program for mycophenolate mofetil. Final minutes incorporate FDA's comments on draft.
June 25, 1993	Syntex notified FDA regarding survival in carcinogenicity studies.
June 28, 1993	Syntex submits updated survival information on carcinogenicity studies.
July 1, 1993	Syntex submits information amendment (Pharmacology/Toxicology reports).
July 2, 1993	Syntex submits background package to FDA for End-of-Phase II meeting with FDA regarding IV formulation.
July 13, 1993	Syntex submits final protocol for a study entitled, "Randomized, Controlled, Dose Ranging Study for the Safety, Pharmacokinetics and

	Efficacy of Intravenous Followed by Oral Mycophenolate Mofetil in the Prevention of Acute Rejection in Primary Cadaveric Renal Allograft Recipients." Syntex also submits documentation of agreements reached with FDA regarding how to proceed with carcinogenicity studies.
August 12, 1993	Syntex submits information amendment (Pharmacology/Toxicology reports).
August 19, 1993	Televideo conference held with FDA (End-of-Phase II meeting for IV formulation).
August 25, 1993	Syntex submits two draft protocols for comment: (1) "An Open Label, Dose Ranging, Pharmacokinetic, Safety, and Tolerance Study of Intravenous Followed by Oral Mycophenolate Mofetil in the Prevention of Rejection in Pediatric Renal and Hepatic Allograft Recipients" and (2) "An Evaluation of the Pharmacokinetics and Bioavailability of Mycophenolate Mofetil Following a 1.5 g IV and Oral Dose in Healthy Volunteers."
September 3, 1993	Syntex notifies the FDA of its intent to discontinue the RA program and submits amended protocols for three ongoing clinical trials.
September 14, 1993	Syntex submits information amendment (Pharmacology/Toxicology reports).
September 21, 1993	5th Annual Report (IND 31,747)
September 23, 1993	Syntex submits draft minutes of the August 19, 1993 video conference which discussed the development program designed to support approval of the IV formulation.
October 4, 1993	1st Annual Report (IND 40,050).
October 11, 1993	Syntex submits a final protocol for a study

	entitled "An Evaluation of the Pharmacokinetics and Bioavailability of Mycophenolate Mofetil Following a 1.5 gm Intravenous and Oral Dose in Healthy Volunteers."
October 21, 1993	FDA sends Syntex a facsimile with comments related to Syntex's plan to suspend development in RA.
October 25, 1993	Syntex submits a background package and requests a meeting with the FDA to discuss the environmental assessment program for mycophenolate mofetil.
October 26, 1993	Syntex submits the final report for the study entitled, "Nine Month Double Blind Placebo Controlled, Multiple Dose Efficacy and Safety of Mycophenolate Mofetil in Patients with Rheumatoid Arthritis."
October 27, 1993	Syntex submits 2 draft protocols for review and comment: (1) "Randomized Double Blind Comparative Study of Mycophenolate Mofetil or Azathioprine Each in Combination with Cyclosporine, or Standard Therapy for Prevention of Rejection After Liver Transplantation" and (2) "An Open Label, Randomized Multiple Dose Study of Mycophenolate Mofetil in Combination with Steroids and Reduced or Standard Dose Cyclosporine or Standard Therapy for Prevention of Rejection After Liver Transplantation."
November 1, 1993	Syntex submits information amendment (Pharmacology/Toxicology reports and updated CMC information).
November 24, 1993	Syntex submits information amendment (Pharmacology/Toxicology reports).

December 20, 1993	Syntex submits draft protocol for study "Bioavailability of Mycophenolate Mofetil Tablet and Capsule Formulations with Varying Dissolution Rates in Healthy Male Subjects." In addition, Syntex submits updated manufacturing and controls information sheets to clarify testing of E. Coli and Salmonella.
December 21, 1993	Syntex submits information amendment (Pharmacology/Toxicology reports).
December 22, 1993	Syntex submits final protocol for pediatric study (see August 25, 1993 entry).
January 5, 1994	Syntex submits draft protocol for review, "Single Dose Pharmacokinetic Drug Interaction Study of Oral Mycophenolate Mofetil and an Oral Contraceptive in Normal Subjects."
January 10, 1994	4th Annual Report (IND 33,872).
January 17, 1994	Syntex submits draft protocol for review, "A Randomized Double-Blind, Multicentre Plasma Concentration Controlled Study of the Safety and Efficacy of Oral Mycophenolate Mofetil When Used in Addition to Cyclosporin and Oral Corticosteroids for the Prevention of Acute Renal Allograft Rejection Episodes."
January 21, 1994	Syntex submits information amendment (Pharmacology/Toxicology reports).
January 26, 1994	Syntex submits request to proceed with cardiac study (draft protocol submitted October 27, 1993).
February 2, 1994	Syntex submits final protocol (see entry of December 20, 1993).
February 9, 1994	Syntex submits background package for meeting requested with FDA to discuss primary endpoint used in renal prevention studies. Syntex also

	submits summary of studies done with mycophenolic acid as well as individual study reports.
February 16, 1994	Syntex submits background package with request for Pre-NDA meeting with FDA to discuss upcoming NDA submissions for mycophenolate mofetil capsules and tablets.
February 21, 1994	Syntex submits draft protocols for review: (1) "Open Label, Randomised Investigation of the Pharmacokinetics of Mycophenolate Mofetil 3g/day Given in Two Divided Doses Orally or as IV Infusions of 1 or 3 Hours Duration or as Continuous Infusion to Renal Allograft Recipients for the Prevention of Rejection" and (2) Investigation of the Pharmacokinetics and Safety of Mycophenolate Mofetil Given by Intravenous Infusion Followed by Oral Dosing to Liver Allograft Recipients."
February 22, 1994	Syntex submits response to FDA comments regarding whether Microbiology section would be included in NDA. Syntex also submits revisions to directions for preparation and infusion of IV formulation.
February 23, 1994	Syntex submits information amendment (Pharmacology/Toxicology reports).
February 25, 1994	Meeting held between Syntex and FDA to discuss choice of primary endpoint used in renal prevention studies and to discuss upcoming NDA submissions for mycophenolate mofetil capsules and tablets.
March 17, 1994	Syntex submits draft minutes of February 25, 1994 meeting for FDA review and comment.
March 18, 1994	Syntex submits draft protocol, "Absorption,

	Metabolism and Excretion of a Single Dose of ^{14}C-Morpholine-Mycophenolate Mofetil Solution in Healthy Volunteers."
March 22, 1994	Pre-NDA meeting held with FDA to discuss format and content of upcoming NDA submissions for mycophenolate mofetil capsules and tablets.
March 25, 1994	Syntex submits letters sent to investigators/pharmacists requesting return of IV clinical supplies.
March 31, 1994	Syntex submits final protocol for study (see January 17, 1994 entry).
April 20, 1994	Syntex meeting with FDA to discuss environmental assessment information to be included in NDA submissions.
April 21, 1994	Syntex submits proposed analysis plans for pooling 1 year patient/graft survival data. Syntex also submits draft minutes of March 22, 1994 Pre-NDA meeting with FDA for review.
April 22, 1994	Syntex submits full CMC information and labels for IV formulation.
April 29, 1994	Syntex submits final protocol for study (see March 18, 1994 entry).
May 11, 1994	Syntex submits updated CMC information for IV formulation.
May 13, 1994	Syntex submits draft minutes of meeting held on April 20, 1994 to discuss environmental assessment issues.
May 20, 1994	Syntex submits information amendment (Pharmacology/Toxicology reports).
May 27, 1994	FDA sends Syntex letter regarding proposed analysis strategy for safety and efficacy.
June 7, 1994	Syntex responds to May 27, 1994 FDA letter regarding statistical analysis issues. Syntex

	also submits new protocol, "Comparative Acceptability of Four Flavor Formulations of Mycophenolate Mofetil Oral Suspension in Healthy Adults."
June 8, 1994	Syntex submits information amendment (Pharmacology/ Toxicology reports).
June 9, 1994	FDA comments on draft minutes of February 25, 1994 and March 22, 1994 meetings received by Syntex.
June 13, 1994	Syntex submits preliminary results of 3 pivotal renal prevention studies.
June 13, 1994	Syntex submits letter regarding CANDA hardware/software that will be delivered to FDA in support of NDA review.
June 14, 1994	Syntex submits final minutes of March 22, 1994 Pre-NDA meeting, and submits updated CMC data for the 250 mg capsule formulation.
June 15, 1994	Syntex further responds to May 27, 1994 FDA letter regarding multiple comparisons procedures.
June 24, 1994	Syntex submits a new protocol for a study entitled "Investigation of the Pharmacokinetics and Safety of Mycophenolate Mofetil Given by Intravenous Infusion Followed by Oral Dosing to Liver Allograft Recipients."
June 29, 1994	Syntex submits final minutes of February 25, 1994 meeting with FDA.
July 6, 1994	Syntex submits information amendment (Pharmacology/Toxicology reports).
July 8, 1994	Syntex responds to FDA request for information regarding patient/graft survival data from renal prevention studies.
July 25, 1994	Syntex submits information amendment

	(Pharmacology/Toxicology reports).
July 26, 1994	Syntex submits a new protocol for a study entitled " Open Label, Randomized Investigation of the Pharmacokinetics of Mycophenolate Mofetil 3 g/day Given in Two Divided Doses Orally of as IV infusions of 1 to 3 Hours Duration or as Continuous infusion to Renal Allograft Recipients for the Prevention of Rejection."
August 11, 1994	Syntex submits preliminary results of ICM 1868 (refractory rejection study).
August 12, 1994	Syntex submits a revision of CMC information for capsule formulation to add tests for product appearance, identity, total aerobic count, E. Coli, Salmonella, Degradation products and loss on drying.
August 16, 1994	6th Annual Report (IND 31,747), 5th Annual Report (IND 33,872), and 2nd Annual Report (IND 40,050).
August 17, 1994	Syntex submits background package for meeting to discuss CMC issues associated with NDA applications.
August 30, 1994	Syntex submits information amendment (Pharmacology/Toxicology reports).
September 8, 1994	Syntex and FDA meeting to discuss CMC issues associated with NDA applications.
September 14, 1994	Syntex submits draft protocol, "A One-Year Randomized, Open-Label, Comparative Trial of Mycophenolate Mofetil or Azathioprine for the Prevention of Acute Renal Allograft Rejection in Uremic Patients with Type I Diabetes Mellitus Receiving Simultaneous Kidney-Pancreas Transplantation."

October 3, 1994	Syntex submits information amendment Pharmacology/Toxicology reports.
October 4, 1994	Syntex submits a change in protocol for the study entitled, "A 36-Month Open Label Extension Study of Mycophenolate Mofetil in Rheumatoid Arthritis Patients", and a change in protocol for a study entitled, "Four Year Open Label Extension Study of Mycophenolate Mofetil in Patients with Rheumatoid Arthritis."
October 17, 1994	Syntex submits proposed analysis plan for 1 year patient/graft survival.
October 19, 1994	FDA comments from CDER labeling and nomenclature committee consult regarding tradename, "CellCept," received by Syntex.
October 21, 1994	Syntex submits final study reports for 3 pivotal renal prevention studies.
November 4, 1994	Syntex receives FDA's comments on draft protocol submitted on September 14, 1994 and proposed analysis plan submitted on October 17, 1994. Syntex submits an updated stability statement to support 250 mg capsule formulations.
November 9, 1994	Syntex submits an NDA application for Mycophenolate Mofetil Tablets and an NDA application for Mycophenolate Mofetil capsules.
November 10, 1994	FDA receives the NDA application for Mycophenolate Mofetil Tablets and an NDA application for Mycophenolate Mofetil capsules.
November 14, 1994	FDA notifies Syntex of receipt of NDA numbers 20-513 (for capsules) and 20-514 (for tablets).
November 15, 1994	Syntex submits response to FDA's comments of November 4, 1994 regarding analysis plan submitted on October 17, 1994.

November 18, 1994	Syntex submits draft protocol, "An Open-Label, Randomized Study of Mycophenolate Mofetil for Treatment of Refractory Acute Cellular Allograft Rejection in Solid Organ Transplant Recipients."
November 21, 1994	Syntex amends NDA to provide patent information in accordance with final rule published October 3, 1994. Syntex also provides description of hardware/software loaned to FDA to facilitate review of NDAs.
November 28, 1994	Telephone conversation with the FDA on the response submitted by Syntex on November 15, 1994 to FDA comments on 1-year survival analysis plan.
December 15, 1994	Syntex amends NDA to provide 1 year patient/graft survival analyses from 3 renal prevention trials.
January 5, 1995	Telephone conversation with the FDA - NDAs deemed fileable.
January 13, 1995	FDA telephones to request information to support inspections of manufacturing sites.
January 24, 1995	FDA comments on draft protocol submitted on November 18, 1994, received by Syntex.
February 1, 1994	Syntex submits final protocol (see November 18, 1994 entry). Syntex also amends NDA with change in resin for child-resistant cap for HDPE bottles.
February 6, 1995	Syntex amends NDA with submission of safety update.
February 21, 1995	Syntex forwards final minutes of September 8, 1994 meeting with FDA to discuss CMC issues.
March 6, 1995	Syntex submits draft presentation materials and background package for Advisory Committee

	hearing March 30, 1995.
March 13, 1995	Syntex and FDA meet to discuss presentations at Advisory Committee hearing.
March 21, 1995	Syntex submits background package for Advisory Committee members.
March 23, 1995	Syntex submits to NDA an electronic copy of the proposed package insert.
March 30, 1995	FDA Advisory Committee hearing to review NDA for mycophenolate mofetil.
April 3, 1995	Syntex amends NDA by submitting a revised label deleting refractory rejection as an indication and the 500 mg tablet dosage form.
April 7, 1995	Syntex meets with the Antiviral Division of the FDA to discuss labeling issues.
April 28, 1995	Syntex amends NDA with submission of final agreed upon labeling.
May 3, 1995	FDA notifies Syntex and signs approval letter for capsules (NDA 50-722 at approval; original number given by the FDA at submission was NDA 20-513)

(12) In the opinion of the applicant, U.S. Patent No. 4,753,935 is eligible for the requested extension.

A. The length of extension claimed is 824 days, and the length of extension claimed is determined as follows:

The regulatory review period - 35 U.S.C. 156(g)(1)(B)

The regulatory review period started on June 24, 1988, the day that IND 31,747 became effective; this was prior to the issuance of U.S. Patent No. 4,753,935 on June 28, 1988. The regulatory review period ended on May 3, 1995, the day that NDA 50-722 was approved. The regulatory review period therefore lasted 2504 days.

The IND period - 35 U.S.C. 156(g)(1)(B)(i)

The period from the effective date of IND 31,747 on June 24, 1988 to the date of receipt by the FDA of NDA 50-722 on November 10, 1994 is 2330 days; one-half of this period is 1165 days. This period is reduced by the number of days in the period that were on or before the date the patent issued, June 28, 1988; so that the period as reduced is 2326 days; one-half of this period as reduced is 1163 days.

The NDA period - 35 U.S.C. 156(g)(1)(B)(ii)

The period from the date of receipt by the FDA of NDA 50-722 on November 10, 1994 to the date of approval of the NDA on May 3, 1995 is 174 days.

Calculation of extension - 35 U.S.C. 156(c)

The maximum possible extension is calculated as the sum of the whole NDA period (174 days) and one-half of the IND period as reduced (1163 days), for a total of 1337 days.

The limitation on extension of 35 U.S.C. 156(c)(3) applies because the term of U.S. Patent No. 4,753,935 remaining after the date of approval of CELLCEPT® (mycophenolate mofetil) 250 mg capsules (4290 days) when added to the regulatory review period as revised (1337 days) is 5627 days, which exceeds fourteen years.

Accordingly, Applicant claims an extension of the term of U.S. Patent No. 4,753,935 of 824 days, so that the patent will expire on May 3, 2009 (fourteen years from the date of approval) when extended.

B. In the alternative, if the Patent and Trademark Office adheres to the "Determination of New Expiration Dates of Certain Patents" published at 60 FR 30069, and permits an extension only on the 17-year from grant term:

The length of extension claimed is 1337 days, and the length of extension claimed is determined as follows:

The regulatory review period - 35 U.S.C. 156(g)(1)(B)

The regulatory review period started on June 24, 1988, the day that IND 31,747 became effective; this was prior to the issuance of U.S. Patent No. 4,753,935 on June 28, 1988. The regulatory review period ended on May 3, 1995, the day that NDA 50-722 was approved. The regulatory review period therefore lasted 2504 days.

The IND period - 35 U.S.C. 156(g)(1)(B)(i)

The period from the effective date of IND 31,747 on June 24, 1988 to the date of receipt by the FDA of NDA 50-722 on November 10, 1994 is 2330 days; one-half of this period is 1165 days. This period is reduced by the number of days in

the period that were on or before the date the patent issued, June 28, 1988; so that the period as reduced is 2326 days; one-half of this period as reduced is 1163 days.

The NDA period - 35 U.S.C. 156(g)(1)(B)(ii)

The period from the date of receipt by the FDA of NDA 50-722 on November 10, 1994 to the date of approval of the NDA on May 3, 1995 is 174 days.

Calculation of extension - 35 U.S.C. 156(c)

The maximum possible extension is calculated as the sum of the whole NDA period (174 days) and one-half of the IND period as reduced (1163 days), for a total of 1337 days.

The term of U.S. Patent No. 4,753,935 will expire, unless extended, on June 28, 2005 (17-year term from grant; see Section 6, first paragraph, of this Application.)

The limitation on extension of 35 U.S.C. 156 (c)(3) does not apply because the term of U.S. Patent No. 4,753,935 remaining after approval of CELLCEPT® (mycophenolate mofetil) 250 mg capsules (3709 days) when added to the regulatory review period as revised (1337 days) is 5046 days, which does not exceed fourteen years.

Accordingly, Applicant claims an extension of the term of U.S. Patent No. 4,753,935 of 1337 days, so that the patent will expire on February 24, 2009 when extended.

C. Applicant reserves the right to assert that the Patent and Trademark Office "Determination" of 60 FR 30069 is incorrect, and that the appropriate patent term and extension of U.S. Patent No. 4,753,935 are those given in Section 12. A. above.

(13) Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought herein.

(14) The fee of $1,000.00 (37 CFR 1.20(j)) is authorized to be charged to Deposit Account No. 19-5430.

(15) Inquiries and correspondence relating to this Application for Patent Term Extension should be directed to:

Pauline Ann Clarke
Patent Law Department, A2-200
Syntex (U.S.A.) Inc.
3401 Hillview Avenue
P.O. Box 10850
Palo Alto, CA 94303.

Telephone enquiries should be addressed to Pauline Ann Clarke at (415)852-1355.

(16) This application for Patent Term Extension is being submitted in duplicate, certified as such below.

(17) The undersigned duly authorized agent for Syntex (U.S.A.) Inc. hereby declares that:

(1) she is a patent attorney authorized to practice before the United States Patent and Trademark Office and has general authority from the Applicant to act on behalf of the Applicant in patent matters;

(2) she has reviewed and understands the contents of this Application;

(3) she believes U.S. Patent No. 4,753,935 is subject to extension pursuant to 37 CFR 1.710;

(4) she believes an extension of the length claimed is justified under 35 U.S.C. 156 and the applicable regulations; and

(5) she believes U.S. Patent No. 4,753,935 meets the conditions for extension of the term of a patent set forth in 37 CFR 1.720.

Respectfully submitted,

Date: June 28, 1995

Pauline Ann Clarke

Pauline Ann Clarke
Reg. No. 29,783
Attorney for Applicant

Patent Law Department, A2-200
Syntex (U.S.A.) Inc.
3401 Hillview Avenue
P.O. Box 10850
Palo Alto, CA 94303

The undersigned hereby certifies that the copy of this Application (together with the appended Attachments A and B) filed herewith is a true and correct copy.

Date: June 28, 1995

Pauline Ann Clarke
Pauline Ann Clarke

PAC/75369

Attachment A

U.S. Patent No. 4,753,935

United States Patent [19]

Nelson et al.

[11] **Patent Number: 4,753,935**

[45] **Date of Patent: Jun. 28, 1988**

[54] **MORPHOLINOETHYLESTERS OF MYCOPHENOLIC ACID AND PHARMACEUTICAL COMPOSITIONS**

[75] Inventors: **Peter H. Nelson**, Los Altos; **Chee-Liang L. Gu**, Sunnyvale; **Anthony C. Allison**; **Elsie M. Eugui**, both of Belmont; **William A. Lee**, Menlo Park, all of Calif.

[73] Assignee: **Syntex (U.S.A.) Inc.**, Palo Alto, Calif.

[21] Appl. No.: **8,717**

[22] Filed: **Jan. 30, 1987**

[51] **Int. Cl.**[4] **A61K 31/535**; C07D 413/12

[52] **U.S. Cl.** **514/233.5**; 514/863; 514/825; 544/153

[58] **Field of Search** 544/153; 514/228, 236

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,868,454 2/1975 Johnson 424/248

OTHER PUBLICATIONS

"Antitumor Activity of Derivatives of Mycophenolic Acid", Suzuki, et al., J. Antibiotics, 29(3), 275–285, 1975.

"A Gastroprotective Anti-Inflammatory Agent: the β-Morpholinoethyl Ester of Niflumic Acid (Morniflumate)", Schiantarelli, et al., Agents and Actions, 14(2), 1984.

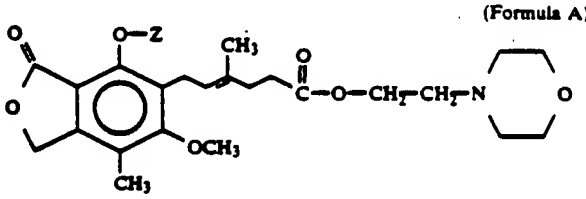
Primary Examiner—Robert W. Ramsuer

Attorney, Agent, or Firm—David A. Lowin; Tom M. Moran

[57] **ABSTRACT**

The compounds and pharmaceutical compositions of Formula A, wherein Z is hydrogen or —C(O)R, where R is lower alkyl or aryl, and the pharmaceutically acceptable salts thereof, are useful as immunosuppressive agents, anti-inflammatory agents, anti-tumor agents, anti-viral agents, and anti-psoriatic agents.

(Formula A)



12 Claims, No Drawings

MORPHOLINOETHYLESTERS OF MYCOPHENOLIC ACID AND PHARMACEUTICAL COMPOSITIONS

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to pharmaceutical compositions, particularly to the morpholinoethyl ester of mycophenolic acid and certain simple ester derivatives of the phenolic hydroxyl group, and to their use as immunosuppressive and anti-inflammatory agents. For example, they are useful for treating rheumatoid arthritis, in which there is an immunologically driven inflammatory process. Because of their effects on purine metabolism, the pharmaceutical compositions of the present invention also find use as anti-tumor, anti-viral and anti-psoriatic agents.

2. Cross-Reference to Related Applications

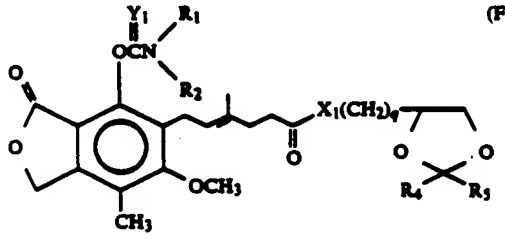
This application is related to Ser. No. 008,909 entitled "Heterocyclic Aminoalkyl Esters of Mycophenolic Acid and Derivatives Thereof," filed contemporaneously herewith; to Ser. No. 803,041, filed Nov. 27, 1985; and to Ser. No. 821,633, filed Jan. 23, 1986.

3. Background Information and Related Disclosures

Inflammatory diseases, in particular rheumatoid arthritis, have been treated with a variety of compounds representing several structural classes and biological activities, including, for example, anti-inflammatory agents (corticosteroids, aspirin, derivatives of arylacetic and arylpropionic acids, and oxicams), immunosuppressive agents and regimes (methotrexate, cyclophosphamide, cyclosporin, and total lymphoid irradiation), and long-acting anti-rheumatic drugs (gold salts, and penicillamine and its derivatives). However, no representative of any of these classes of compounds is regarded as ideal.

Mycophenolic acid is a weakly-active antibiotic found in the fermentation broth of *Penicillium brevicompactum*. Some compounds relating to mycophenolic acid, and their uses in the treatment of inflammatory diseases, such as rheumatoid arthritis, are disclosed in the following two prior related applications.

Ser. No. 803,041, filed Nov. 27, 1985, relates to compounds having the general structure of Formula 1:



(Formula 1)

and the pharmaceutically acceptable salts thereof, where:

R_1 is H or lower alkyl having 1 to 6 carbon atoms;

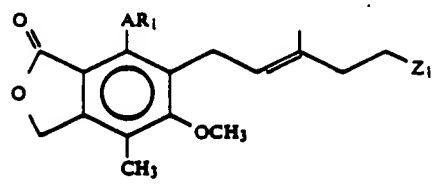
R_2 is H, lower alkyl having 1 to 6 carbon atoms or -phenyl-4-CO_2R_3, in which R_3 is H, lower alkyl having 1 to 6 carbon atoms or a pharmaceutically acceptable cation;

R_4 and R_5 are each independently H or lower alkyl having 1 to 6 carbon atoms;

X_1 and Y_1 are each independently O or S; and

q is an integer of 1–6.

Ser. No. 821,633, filed Jan. 23, 1986, relates to compounds having the general structure of Formula 2:

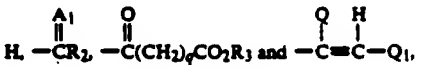


(Formula 2)

and the pharmaceutically acceptable salts thereof, where:

A is oxygen or sulfur;

R_1 is selected from the group consisting of:



in which:

A_1 is oxygen or sulfur;

q is an integer from 0–6;

R_2 is alkyl, haloalkyl or $-NR_4R_5$, where:

R_4 and R_5 are independently H, alkyl, haloalkyl, cycloalkyl, phenyl optionally monosubstituted with halogen, hydroxy, carboxy, chlorocarbonyl, sulfonylamino, nitro, cyano, phenyl, alkyl, acyl, alkoxycarbonyl, acylamino, dialkylamino or dialkylaminoethoxycarbonyl, phenyl optionally disubstituted with hydroxy, carboxy, nitro or alkyl, or benzyl optionally substituted with dialkylamino;

R_3 is H, alkyl or a pharmaceutically acceptable cation;

Q and Q_1 are independently H or $-CO_2R_3$; and

Z_1 is selected from the group consisting of: 1H-tetrazolyl, $-CH_2OH$, $-CHO$, $-CN$, $-C(O)A_2R_6$ and $-C(O)NR_7R_8$, in which:

A_2 is oxygen or sulfur;

R_6 is H, alkyl, alkenyl, cycloalkyl, optionally substituted phenyl, optionally substituted benzyl or a pharmaceutically acceptable cation; and

R_7 and R_8 are independently H, alkyl or cycloalkyl, or R_7 and R_8 taken together are $-(CH_2)_2O(CH_2)_2-$, $-(CH_2)_4-$, or $-(CH_2)_5-$;

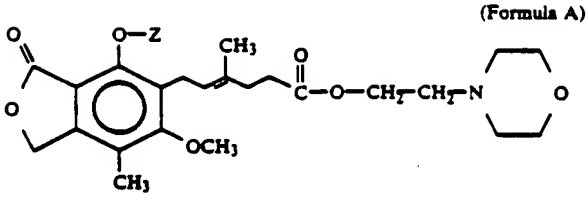
with the proviso that R_1 and R_6 cannot both be H if A and A_2 are oxygen.

Compounds somewhat structurally similar to the compounds of Formulae 1 and 2 are described in U.S. Pat. Nos. 3,705,894; 3,853,919; 3,868,454; 3,880,995, in Japanese Pat. No. J 57024380, in *J. Antibiot.*, 29(3), 275–85, 286–91 (1976), and in *Cancer Research*, 36(8), 2923–7 (1976). The disclosed compounds are described as having anti-tumor, immunosuppressive, anti-viral, anti-arthritic and/or anti-psoriastic activities.

SUMMARY OF THE INVENTION

One aspect of the present invention concerns the morpholinoethyl ester of mycophenolic acid and certain derivatives of mycophenolic acid, i.e. compounds having the structure of Formula A, which follows:

(Formula A)



wherein Z is hydrogen or —C(O)R,

where R is lower alkyl or aryl, and the pharmaceutically acceptable salts thereof.

In another aspect, the invention relates to a pharmaceutical composition containing a therapeutically effective amount of a compound of Formula A admixed with at least one pharmaceutically acceptable excipient.

In still another aspect, the invention relates to a method of treating autoimmune disorders, psoriasis, inflammatory diseases including in particular rheumatoid arthritis, and for treating tumors and viruses in a mammal by administering to a mammal in need of such treatment a therapeutically effective amount of a compound of Formula A.

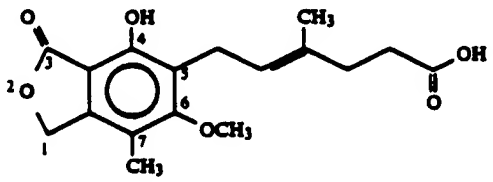
Compounds of Formula A have advantageous pharmacokinetic properties, for example, solubility in the delivery environment (e.g., the stomach), peak plasma concentration, maximum plasma concentration, and improved activity, e.g., anti-inflammatory activity as compared to mycophenolic acid.

DETAILED DESCRIPTION OF THE INVENTION

Definitions and General Parameters

The following definitions are set forth to illustrate and define the meaning and scope of the various terms used to describe the invention herein.

The numbering of the mycophenolic acid is as follows:



The compounds of the invention will be named using the above-shown numbering systems as the morpholinoethyl esters of E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid and its derivatives. The compounds of the present invention are prepared as the E (or Entgegen) position isomer. Some representative compounds are named as follows:

the compound of Formula A where Z is —C(O)R and wherein R is methyl is named "morpholinoethyl E-6-(1,3-dihydro-4-acetoxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate" and

the compound of Formula A where Z is —C(O)R and wherein R is phenyl is named "morpholinoethyl E-6-(1,3-dihydro-4-benzoyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate."

As used herein, the term "alkyl" refers to a fully saturated monovalent radical containing only carbon and hydrogen, and which may be a cyclic, branched or straight chain radical. This term is further exemplified by radicals such as methyl, ethyl, t-butyl, pentyl, heptyl and pivalyl.

The term "lower alkyl" refers to a monovalent alkyl radical of one to six carbon atoms. This term is further exemplified by such radicals as methyl, ethyl, n-propyl, isopropyl, N-butyl, t-butyl, i-butyl (or 2-methylpropyl), isoamyl, pentyl, and isopentyl.

The term "aryl" refers to a substituted or unsubstituted monovalent unsaturated aromatic carbocyclic radical having a single ring (e.g., phenyl) or two condensed rings (e.g., naphthyl).

The term "acyl" refers to a radical based on an organic acid, e.g., —C(O)R^1 where R^1 is alkyl or aryl.

As used herein, the term "halo" refers to fluoro, bromo, chloro and iodo.

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin layer chromatography or thick-layer chromatography, or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be found by reference to the examples hereinbelow. However, other equivalent separation or isolation procedures can, of course, also be used.

A "pharmaceutically acceptable salt" may be any salt derived from an inorganic or organic acid. The term "pharmaceutically acceptable anion" refers to the anion of such salts. The salt and the anion are chosen not to be biologically or otherwise undesirable. These salts are formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid (giving the sulfate and bisulfate salts), nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like.

As used herein, the term "treatment" or "treating" means any treatment of a disease in a mammal, and includes:

(i) preventing the disease, that is, causing the clinical symptoms of the disease not to develop;

(ii) inhibiting the disease, that is, arresting the development of clinical symptoms; and/or

(iii) relieving the disease, that is, causing the regression of clinical symptoms.

Unless specified to the contrary, the reactions described herein take place at atmospheric pressure over a temperature range from about 10° C. to about 100 C., more preferably from about 10° C. to about 50° C., and most preferably at about room temperature.

Preparation of the Compounds of Formula A

The compounds of Formula A can be prepared according to several synthetic pathways, depending upon the substitution at Z, typically starting with mycophenolic acid, which is commercially available. Where Z is —C(O)R, the phenolic oxygen of mycophenolic acid can be acylated either before or after the esterification of the acid. Where Z is hydrogen, the starting material is typically mycophenolic acid.

Morpholinoethyl Esterification of Mycophenolic Acids

Many standard esterification procedures may be used, for example, as described in *Synthetic Organic Chemistry* by R. B. Wagner and H. D. Zook (Wiley, New York) 1956, see pages 479–532. Two presently preferred synthetic routes are described below for conversion of mycophenolic acid and its derivatives into the morpholinoethyl ester compounds of Formula A. The first route involves conversion into an acid halide, followed by condensation with morpholinoethanol to the end product. The second route involves conversion directly into the end product using a carbodiimide reaction.

As an example, a less preferred third route entails starting with an ester of mycophenolic acid (other than the morpholinoethyl ester) in an ester exchange reaction for conversion into the desired end product.

The Acid Halide-Condensation Route

In the first synthetic route, mycophenolic acid or an acylated derivative thereof is dissolved or suspended in a solvent inert under the conditions of the reaction (i.e., an inert solvent, such as benzene, toluene, acetonitrile, tetrahydrofuran, diethyl ether, chloroform or preferably methylene chloride) and an excess (about 10 molar equivalents to 1) of a halogenating agent (e.g., thionyl chloride) is added, optionally together with a small amount of dimethylformamide. The reaction mixture is stirred for about 1–8 hours, preferably about 4 hours, to yield the corresponding acid halide.

The acid halide is dissolved in an inert solvent, as described above, and reacted by a condensation reaction with a cooled solution (e.g., maintained at about 4° C.) of morpholinoethanol [also named as 4-(2-hydroxyethyl)morpholine], to which it is added slowly over a period of about 10 minutes to 2 hours, preferably about 90 minutes. The end product of Formula A is isolated and purified by conventional procedures.

The Carbodiimide Route

In the second synthetic route, mycophenolic acid or an acylated derivative thereof is dissolved in a solvent inert under the conditions of the reaction [such as dry tetrahydrofuran ("THF"), dichloromethane, or carbon tetrachloride; preferably THF] and reacted with morpholinoethanol in the presence of a carbodiimide, such as DCC ("dicyclohexylcarbodiimide") or di-p-tolylcarbodiimide. The molar ratio of alcohol to the starting acid is about 1:1. The reaction takes place at atmospheric pressure over a period of about 4–8 hours, preferably over 6 hours. A temperature range from about 10° C. to about reflux temperature, preferably about room temperature may be used. The end product of Formula A is isolated and purified in the usual manner.

Acylation of the Phenolic Oxygen

The compounds of Formula A where Z is —C(O)R are prepared by dissolving mycophenolic acid or the morpholinoethyl ester thereof in an inert organic solvent as defined above (e.g., acetonitrile or preferably pyridine) and reacting it with about 1 to 6 molar equivalents, preferably about 3 molar equivalents, of the appropriate acyl halide or anhydride (e.g., acetic anhydride, propionyl chloride or pivaloyl chloride) in the presence of about 1 to 6 molar equivalents, preferably about 3 molar equivalents, of an inorganic base (such as sodium carbonate, potassium bicarbonate or the like) or a tertiary organic base (such as triethylamine, N-methylpiperidine or preferably pyridine). Certain bases (e.g., pyridine) can also serve as the inert organic solvent. The reaction takes place at a temperature of about 0°–25° C., preferably about 5° C., for about 1–10 hours, preferably about 3 hours. When the reaction is substantially complete, the acylated product is isolated by conventional means.

Salts of Compounds of Formula A

The compounds of Formula A may be converted to corresponding acid addition salts. The conversion is accomplished by treatment with at least a stoichiometric amount of an appropriate acid, such as hydrochloric acid, sulfuric acid, methanesulfonic acid or the like. Typically, the free base is dissolved in a polar organic solvent such as ethanol, methanol, or ethyl acetate and the acid added in water, ethanol, methanol, or isopropanol. The temperature is maintained at 0°–50° C. The resulting salt precipitates spontaneously or may be brought out of solution with a less polar solvent.

A dibasic acid, such as sulfuric acid, can form two salts with the compounds of this invention. One such salt, in which one mole of the base and one mole of the acid are present, is called the bisulfate (or hydrogen sulfate) salt. The other, in which two moles of the base and one mole of the acid are present, is called the sulfate.

The acid addition salts of the compounds of Formula A may be decomposed to the corresponding free bases by treating with an excess of a suitable base, such as ammonia or sodium bicarbonate, typically in the presence of aqueous solvent, and at a temperature of between 0° and 50° C. The free base form is isolated by conventional means, such as extraction with an organic solvent.

Preferred Compounds

Most preferred are the compound of Formula A where Z is hydrogen, i.e., morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate, and its pharmaceutically acceptable salts (preferably the hydrochloride, sulfate and bisulfate salts).

Also preferred are the following compounds and pharmaceutically acceptable salts (preferably the hydrochloride, sulfate and bisulfate salts) of Formula A where Z is —C(O)R:

morpholinoethyl E-6-(1,3-dihydro-4-acetoxy-6-methoxy-7-methyl-3-oxo-5-isobenzylfuranyl)-4-methyl-4-hexenoate;

morpholinoethyl E-6-(1,3-dihydro-4-propionyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate;

morpholinoethyl E-6-(1,3-dihydro-4-pivaloyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate; and

morpholinoethyl E-6-(1,3-dihydro-4-benzoyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.

Preferred Processes

The compounds of the present invention can be prepared according to the following last steps:

an E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoyl halide, is condensed with morpholinoethanol to give a compound according to Formula A where Z is hydrogen;

an E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid is contacted with morpholinoethanol in the presence of a carbodiimide to give a compound according to Formula A where Z is hydrogen;

an E-6-(1,3-dihydro-4-acyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoyl halide, is condensed with morpholinoethanol to give a compound according to Formula A where Z is —C(O)R;

an E-6-(1,3-dihydro-4-acyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid is condensed with morpholinoethanol in the presence of a carbodiimide to give a compound according to Formula A where Z is —C(O)R;

morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate is condensed with an acyl halide or anhydride to give a compound according to Formula A where Z is —C(O)R;

contacting a pharmaceutically acceptable acid with a compound of Formula A to form the corresponding acid addition salt of Formula A;

substituting a pharmaceutically acceptable acid salt of Formula A with another pharmaceutically acceptable acid; and

contacting an acid addition salt of Formula A with a base to form the corresponding free base compounds of Formula A.

Utility and Administration

General Utility

The compounds of the present invention, including the pharmaceutically acceptable salts thereof, and the compositions containing them, are useful as immunosuppressive agents, anti-inflammatory agents, anti-tumor agents, anti-viral agents, and anti-psoriatic agents in mammals, whether domestic (cattle, pigs, sheep, goats, horses), pets (cats, dogs), or preferably humans. For example compounds of Formula A are useful for treating rheumatoid arthritis, in which there is an immunologically driven inflammatory process. These compounds can be used both prophylactically (e.g., to prevent allograft rejection) and therapeutically.

Testing

Initial animal screening tests to determine anti-inflammatory activity potential include the adjuvant arthritis assay according to the method of Pearson, Proc. *Soc. Exp. Biol. Med.*, 91: 95–101 (1956).

Also, in vitro tests, for example those using synovial explants from patients with rheumatoid arthritis, Dayer, et al., *J. Exp. Med.*, 145: 1399–1404 (1977), are useful in determining whether compounds exhibit anti-inflammatory activity.

Autoimmune activity is determined utilizing experimental allergic encephalomyelitis by a modification of a procedure initially described by Grieg, et al., *J. Pharmacol. Exp. Ther.* 173: 85 (1970).

Immunosuppressive activity is determined by both in vivo and in vitro procedures. In vivo activity is determined utilizing a modification of the Jerne hemolytic plaque assay, [Jerne et al., "The agar plaque technique for recognizing antibody producing cells," *Cell-bound Antibodies*, Amos, B. and Kaprowski, H. editors (Wistar Institute Press, Philadelphia) 1963, p. 109]. In vitro activity is determined by an adaptation of the procedure described by Greaves, et al. ["Activation of human T and B lymphocytes by polyclonal mitogens," Nature. 248, 698–701 (1974)].

Anti-viral activity is determined by the procedure described by Smee, et al. ["Anti-Herpesvirus Activity of the Acyclic Nucleoside 9-(1,3-Dihydroxy-2-Propoxymethyl)Guanine," Antimicrobial Agents and Chemotherapy, 23 (5), 676–682 (1983)] or as described by Planterose ["Antiviral and cytotoxic effects of mycophenolic acid," *Journal of General Virology*, 4, 629 (1969)].

Tests for systemic activity in psoriasis can be carried out as described by Spatz, et al. "Mycophenolic acid in psoriasis," *British Journal of Dermatology*, 98, 429 (1978)].

Tests for anti-tumor activity can be performed as described by Carter, et al. ["Mycophenolic acid: an anticancer compound with unusual properties," Nature , 223, 848 (1969)].

General Administration

Administration of the active compounds of Formula A, in pure form or in an appropriate pharmaceutical composition can be carried out via any of the accepted modes of administration of agents for serving similar utilities. Thus, administration can be, for example, orally, nasally, parenterally or topically, in the form of solid, semi-solid, lyophilized powder, or liquid dosage forms, such as for example, tablets, suppositories, pills, capsules, powders, solutions, suspensions, emulsions, creams, lotions, aerosols, ointments or the like, preferably in unit dosage forms suitable for simple administration of precise dosages. The compositions will include a conventional pharmaceutical carrier or excipient and an active compound of Formula A and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, etc.

Generally, depending on the intended mode of administration, the pharmaceutically acceptable compositions will contain about 1% to about 99% by weight of the pharmaceutically active compound of this invention and 99% to 1% by weight of suitable pharmaceutical excipients. Preferably, the composition will be about 5 to 75% by weight of a pharmaceutically active compound, with the rest being suitable pharmaceutical excipients.

The preferred manner of administration, for the conditions detailed above, is oral using a convenient daily dosage regimen which can be adjusted according to the degree of affliction. For such oral administration, a pharmaceutically acceptable, non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, gelatin, sucrose, magnesium carbonate, and the like. Such compositions take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations and the like.

Preferably the compositions will take the form of a pill or tablet and thus the composition will contain, along with the active ingredient, a diluent such as lactose, sucrose, dicalcium phosphate, and the like; a disintegrant such as starch or derivatives thereof; a lubricant such as magnesium stearate and the like; and a binder such as a starch, gum acacia, polyvinylpyrrolidone, gelatin, cellulose and derivatives thereof, and the like.

The active compounds of Formulas I may be formulated into a suppository using, for example, about 0.5%

to about 50% active ingredient disposed in a carrier of polyethylene glycols (PEG) [e.g., PEG 1000 (96%) and PEG 4000 (4%)].

Liquid pharmaceutically administerable compositions can, for example, be prepared by dissolving, dispersing, etc. an active compound (about 0.5% to about 20%), as described above, and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol and the like, to thereby form a solution or suspension.

If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc.

Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington's Pharmaceutical Sciences*, 16th Ed., (Mack Publishing Company, Easton, Pa., 1980). The composition to be administered will, in any event, contain a quantity of the active compound(s) in a pharmaceutically effective amount for relief of the particular condition being treated when administered in accordance with the teachings of this invention.

Generally, the compounds of the invention are administered in a therapeutically effective amount, i.e., a dosage sufficient to effect treatment, which will vary depending on the individual and condition being treated. Typically, a therapeutically effective daily dose is from 0.02 to 100 mg/kg of body weight per day of an active compound of Formula A. Most conditions respond to treatment comprising a dosage level on the order of 0.4 to 30 mg/kg of body weight per day, and most preferably about 10 mg/kg/day. Thus, for administration to a 70 kg person, the dosage range would be about 1.4 mg to 7 g per day, preferably about 7.0 to 700 mg per day.

EXAMPLES

The following examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. They should not be considered as a limitation on the scope of the invention, but merely as being illustrative and representative thereof.

EXAMPLE 1

Morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate

1A. Formula A where Z is Hydrogen

E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid (mycophenolic acid) (32.0 g) was dissolved in dichloromethane (250 ml), followed by the addition of thionyl chloride (25.0 ml) and dimethylformamide (0.3 ml). The reaction mixture was stirred at room temperature for 3 hours, after which the volatile components were removed under vacuum to afford E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoic acid chloride as an oil.

A solution of morpholinoethanol (30.5 ml) in dichloromethane (250 ml) was chilled to 4° C. on an ice bath. The mycophenolic acid chloride oil was dissolved in dichloromethane (50.0 ml) and added to the chilled solution. After stirring for 90 minutes (at 4° C.), the reaction mixture was washed with water and then with aqueous sodium bicarbonate. The organic solution was dried with sodium sulphate and evaporated to yield morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate (m.p. 93°-94° C.).

EXAMPLE 2

Morpholinoethyl E-6-(1,3-dihydro-4-acetoxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate

2A. Formula A where Z is $-C(O)CH_3$

Morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate (10.0 g) was dissolved in pyridine (50.0 ml) followed by the addition of acetic anhydride (10.0 ml). The mixture was stirred at room temperature for 90 minutes, then poured into water and extracted with ethyl acetate. The organic solution was dried and evaporated to give morpholinoethyl E-6-(1,3-dihydro-4-acetoxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.

2B. Formula A where Z is Other Than $-C(O)CH_3$

Similarly, by following the procedure of part A above and substituting for acetic anhydride the following materials:

propionyl chloride,
2-methylpropionyl chloride,
pivaloyl chloride, and
benzoyl bromide;

there are obtained the following respective compounds:

morpholinoethyl E-6-(1,3-dihydro-4-propionyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate,

morpholinoethyl E-6-[1,3-dihydro-4-(2-methylpropionyloxy)-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl]-4-methyl-4-hexenoate,

morpholinoethyl E-6-(1,3-dihydro-4-pivaloyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate, and

morpholinoethyl E-6-(1,3-dihydro-4-benzoyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate.

EXAMPLE 3

Morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride

3A. Hydrochloride Salt of Formula A where Z is Hydrogen

Morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate (38.0 g) was dissolved in isopropanol (200 ml) and the solution was added to a solution of hydrogen chloride (10.0 g) in isopropanol (150 ml). The hydrochloride salt was collected by filtration and dried under vacuum (m.p. 154°-155° C.).

3B. Hydrochloride Salts of Formula A where Z is Other Than $-C(O)CH_3$

Similarly, by following the procedure of part A above and substituting for morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate the following materials (prepared, e.g., as in Example 2B):

morpholinoethyl E-6-(1,3-dihydro-4-propionyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate,

morpholinoethyl E-6-[1,3-dihydro-4-(2-methylpropionyloxy)-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl]-4-methyl-4-hexenoate,
morpholinoethyl E-6-(1,3-dihydro-4-pivaloyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate, and
morpholinoethyl E-6-(1,3-dihydro-4-benzoyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate; there are obtained the following respective compounds:
morpholinoethyl E-6-(1,3-dihydro-4-propionyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride (m.p. 140°–144° C.),
morpholinoethyl E-6-[1,3-dihydro-4-(2-methylpropionyloxy)-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl]-4-methyl-4-hexenoate hydrochloride,
morpholinoethyl E-6-(1,3-dihydro-4-pivaloyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride (m.p. 135°–139° C.), and
morpholinoethyl E-6-(1,3-dihydro-4-benzoyloxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

EXAMPLE 4

Morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate bisulfate

4A. Bisulfate Salt of Formula A where Z is Hydrogen

Morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate (4.6 g) was dissolved in ethyl acetate (50 ml) and the solution was added to a solution of sulfuric acid (1.25 g) in isopropanol (50 ml). The bisulfate salt was collected by filtration, washed with ethyl acetate and dried under vacuum at 50° C. (m.p. 143°–145° C.).

4B. Bisulfate Salts of Formula A where Z is Other Than —C(O)CH_3

Similarly, by following the procedure of part A above and substituting for morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate the materials prepared in Example 2B, the corresponding bisulfate salts are obtained.

EXAMPLE 5

This example illustrates the preparation of a representative pharmaceutical formulation for oral administration containing an active compound of Formula A, e.g., morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

Ingredients	Quantity per tablet, mgs.
Active compound	200
lactose, spray-dried	148
magnesium stearate	2

The above ingredients are mixed and introduced into a hard-shell gelatin capsule.

Other compounds of Formula A, such as those prepared in accordance with Examples 2–4, can be used as the active compound in the preparation of the orally administrable formulations of this example.

EXAMPLE 6

This example illustrates the preparation of another representative pharmaceutical formulation for oral administration, containing an active compound of Formula A, e.g., morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

Ingredients	Quantity per tablet, mgs.
Active compound	400
cornstarch	50
lactose	145
magnesium stearate	5

The above ingredients are mixed intimately and pressed into single scored tablets.

Other compounds of Formula A, such as those prepared in accordance with Examples 2–4, can be used as the active compound in the preparation of the orally administrable formulations of this example.

EXAMPLE 7

This example illustrates the preparation of a representative pharmaceutical formulation containing an active compound of Formula A, e.g., morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

An oral suspension is prepared having the following composition:

Ingredients	
Active compound	1.0 g
fumaric acid	0.5 g
sodium chloride	2.0 g
methyl paraben	0.1 g
granulated sugar	25.5 g
sorbitol (70% solution)	12.85 g
Veegum K (Vanderbilt Co.)	1.0 g
flavoring	0.035 ml
colorings	0.5 mg
distilled water q.s. to	100 ml

Other compounds of Formula A, such as those prepared in accordance with Examples 2–4, can be used as the active compound in the preparation of the orally administrable formulations of this example.

EXAMPLE 8

This example illustrates the preparation of a representative pharmaceutical formulation containing an active compound of Formula A, e.g., morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

An injectable preparation buffered to a pH of 4 is prepared having the following composition:

Ingredients	
Active compound	0.2 g
Sodium Acetate Buffer Solution (0.4 M)	2.0 ml
HCl (1N) q.s. to	pH 4
water (distilled, sterile) q.s. to	20 ml

Other compounds of Formula A, such as those prepared in accordance with Examples 2–4, can be used as

the active compound in the preparation of the injectable formulations of this example.

EXAMPLE 9

This example illustrates the preparation of a representative pharmaceutical formulation for topical application containing an active compound of Formula A, e.g., morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

Ingredients	grams
Active compound	0.2–10
Span 60	2
Tween 60	2
Mineral oil	5
Petrolatum	10
Methyl paraben	0.15
Propyl paraben	0.05
BHA (butylated hydroxy anisole)	0.01
Water q.s. to	100

All of the above ingredients, except water, are combined and heated to 60° C. with stirring. A sufficient quantity of water at 60° C. is then added with vigorous stirring to emulsify the ingredients, and water then added q.s. 100 g.

Other compounds of Formula A, such as those prepared in accordance with Examples 2–4, can be used as the active compound in the preparation of the topical formulations of this example.

EXAMPLE 10

This example illustrates the preparation of a representative pharmaceutical formulation containing an active compound of Formula A, e.g., morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride.

A suppository totalling 2.5 grams is prepared having the following composition:

Active compound	500 mg
witepsol H-15*	balance

(*triglycerides of saturated vegetable fatty acid; a product of Riches-Nelson, Inc., New York, N.Y.).

Other compounds of Formula A, such as those prepared in accordance with Examples 2–4, can be used as the active compound in the preparation of the suppository formulations of this example.

EXAMPLE 11

Determination of Anti-Inflammatory Activity Utilizing Adjuvant-Induced Arthritis In The Rat

Protocol:

This procedure is a modification of a procedure initially described by Pearson, C. M., Proc. Soc. Exp. Biol. Med., 91: 95–101 (1956).

Female Simonsen albino rats weighing 160–180 g receive 0.1 ml of a suspension in paraffin oil of heat-killed M. *Mycobacterium butyricum* (10 mg/ml) by means of an intradermal injection into the proximal ⅓ of the tail on day 0. Beginning on day 1, the test material is administered orally in an aqueous vehicle (0.5 ml/dose) twice each day for 17 days. On day 18 the intensity of the swelling of the four foot pads and tail is determined utilizing a scoring system in which the swelling in the four paws was scored 0–4 for each paw and the tail swelling is scored 0–3, such that the total maximum score is 19.

The compounds of the present invention show anti-inflammatory activity when tested by this method.

EXAMPLE 12

Determination of Autoimmune Activity Utilizing Experimental Allergic Encephalomyelitis

Protocol:

This procedure is a modification of a procedure initially described by Grieg, et al., *J. Pharmacol. Exp. Ther.* 173: 85 (1970).

On day 1, Experimental Allergic Encephalomyelitis is induced by giving an 0.1 ml sub-plantar injection into the dorsum of the right hind paw of an emulsion consisting of 15 mg (wet weight) of syngeneic spinal cord tissue, 0.06 ml of Freund's Incomplete Adjuvant (Difco), 0.04 ml of sterile 0.9% saline, and 0.2 mg of heat killed and dried *Mycobacterium butyricum* (Difco). On days 12–17, clinical evaluations are obtained for each animal. The animals are considered positive if flaccid hind limb paralysis is present on one or more days.

The compounds of the present invention show autoimmune activity when tested by this method.

EXAMPLE 13

Determination of Immunosuppressive Activity Utilizing The Hemolytic Plaque Forming Cell Assay

This procedure is a modification of "The agar plaque technique for recognizing antibody producing cells," a procedure initially described by Jerne, et al. [Cell-bound Antibodies, Amos and Kaprowski editors (Wistar Institute Press, Philadelphia, 1963), p. 109].

Groups of 5–6 adult C578B1/6 male mice were sensitized with 1×10^8 sheep red blood cells ("SRBC") and simultaneously treated with an oral dosage form of the test material in an aqueous vehicle. Animals in a control group receive the same volume of vehicle. Four days after SRBC inoculation, spleens are dispersed in loose Ten Broeck homogenizers. The number of nucleated cells ("WBC") is determined and the spleen cell suspension is mixed with SRBC, guinea pig complement and agar solution at 0.5% concentration. Aliquots of the above mixture (0.1 ml) are dropped on four separate quadrants of a Petri dish and are covered with cover slips. After two hours incubation at 37° C., areas of hemolysis around plaque-forming cells ("PFC") are counted with a dissecting microscope. Total WBC/spleen, PFC/spleen and PFC/10^6 WBC ("PPM") are calculated for each mouse spleen. Geometric means of such treatment group are then compared with the vehicle-treated control group.

The compounds of the present invention show immunosuppressive activity when tested by this method.

EXAMPLE 14

Determination of Immunosuppressive Activity Utilizing Responses of Human Peripheral Blood Lymphocytes to T- and B-cell Mitogens

This procedure is a modification of a procedure initially described by Greaves, et al. ["Activation of human T and B lymphocytes by polyclonal mitogens," Nature, 248, 698–701 (1974)].

Human mononuclear cells ("PBL") are separated from heparinized whole blood by density gradient cen-

trifugation in Ficoll-Paque (Pharmacia). After washing, 2×10^5 cells/well are cultured in microtiter plates with RPMI 1640 supplemented with 5% fetal calf serum, penicillin and streptomycin. To evaluate differential effects on T- and B-lymphocytes, different mitogens are used: PHA (Sigma) at 10 μg/ml, PWM (Sigma) at 20 μg/ml and Staphylococcus Protein A bound to Sepharose (SPA) (Sigma) 2 mg/ml or 14 μg/ml of Protein A. Test materials are tested at concentrations between 10^4 and 10^8M, by addition to the culture at time 0. Cultures are set up in quadruplicate and incubated at 37° C. in a humidified atmosphere with 7% CO_2 for 72 hours. A pulse of 0.5 μCi/well of ^{3}H-thymidine is added for the last 6 hours. Cells are collected on glass fiber filters with an automatic harvester and radioactivity is measured by standard scintillation procedures. The 50% inhibitory concentration ("IC_{50}") for mitogenic stimulation is determined graphically.

The compounds of the present invention show immunosuppressive activity when tested by this method.

EXAMPLE 15

Determination of Anti-viral Activity Utilizing 50% Plaque Reduction Assay

This procedure is described by Smee, et al., in "Anti-Herpesvirus Activity of the Acyclic Nucleoside 9-(1,3-Dihydroxy-2-Propoxymethyl)Guanine" [*Antimicrobial Agents and Chemotherapy*, 23(5), 676–682 (1983)].

Confluent monolayers of Vero cells in six-well Costar microplates (Bellco Glass, Inc., Vineland, N.J.) are infected with 100 to 200 PFU of HSV or pseudorabies virus. After a 1.25 hour adsorption period, the virus is aspirated and EMEM containing 0.6% methylcellulose, 2% fetal bovine serum, 0.25% $NaHCO_3$, 10 mM HEPES buffer, 50 μg of gentamicin per ml, and the test compound are applied. Three wells per dilution of the test compound, and six control wells without test compound are incubated for four days at 37° C. in 5% CO_2, after which the methylcellulose layer is removed and the cells are fixed with methanol for 10 minutes and stained with 10% Giemsa stain (Fisher Scientific Co., Fair Lawn, N.J.) for 20 minutes. After the plates are aspirated and dried, the plaques are counted at 13× magnification with a Bellco plaque viewer. Drug concentrations that reduced plaque numbers by 50% [the 50% inhibitory dose (ID_{50})] are calculated, e.g., with a computer using a semilog probit analysis program [see Finney, D. J., *Probit analysis*, 3rd Ed., p. 333, (Cambridge University Press, London, 1971)].

The compounds of the present invention show antiviral activity when tested by this method.

EXAMPLE 16

Bioavailability—Plasma Levels

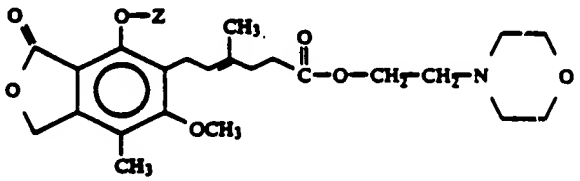
Compounds of Formula A are given to four male cynomolgus monkeys as a solid dosage form (about 20 mg/kg body weight) with one-week intervals between doses. Mycophenolic acid is given to a control group. The compounds are weighed into hard gelatin capsules and administered orally. Samples of plasma are obtained at 0.25, 0.5, 1, 3, 5, 7 and 24 hours after dosing, and are analyzed for concentrations of mycophenolic acid by HPLC.

Compounds of the present invention [morpholinoethyl E-6-(1,3-dihydro-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride and morpholinoethyl E-6-(1,3-dihydro-4-acetoxy-6-methoxy-7-methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate hydrochloride] were compared to mycophenolic acid according to the above protocol. The compounds of the present invention demonstrated faster absorption to higher peak plasma levels than mycophenolic acid.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

What is claimed is:

1. A compound represented by the formula:



wherein:

Z is hydrogen or —C(O)R,

where R is lower alkyl or aryl; or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1 wherein Z is hydrogen.

3. The hydrochloride salt of the compound of claim 2.

4. The sulfate or bisulfate salt of the compound of claim 2.

5. The bisulfate salt of the compound of claim 2.

6. The compound of claim 1 where R is lower alkyl.

7. The compound of claim 6 wherein Z is —C-(O)CH_3.

8. The hydrochloride salt of the compound of claim 7.

9. The compound of claim 6 wherein Z is —C-(O)CH_2CH_3.

10. The compound of claim 6 wherein Z is pivaloyloxy.

11. The compound of claim 1 wherein Z is benzoyloxy.

12. A pharmaceutical composition comprising a pharmaceutically acceptable non-toxic excipient and a therapeutically effective amount of a compound of claim 1.

* * * * *

Attachment B

Maintenance Receipt for U.S. Patent No. 4,753,935

SYNTEX (U.S.A.) INC.
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LEGAL AFFAIRS DIVISION

356 2252
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Re: MAINTENANCE FEE AUTHORIZATION

The data shown below reflects MAINTENANCE FEES and any necessary surcharges which should be PAID for the patents listed. This letter authorizes that payment be made and all required fees be charged to Deposit Account No. 19-5430.

Patent No.	Fee Code	Fee Amount	Serial No.	Patent Dt.	File Dt.	Attorney Docket & Code
4452775	174	$1670.00	446749	06/05/84	12/03/82	23520
4454151	171	$ 495.00	360754	06/12/84	03/22/82	22790
4457941	171	$ 495.00	360753	07/03/84	03/22/82	22780
4458081	171	$ 495.00	387564	07/03/84	06/11/82	21950 DIV 1
4466981	174	$1670.00	437063	08/21/84	10/27/82	23420
4749804	173	$ 830.00	872561	06/07/88	06/10/86	22610 DIV 1
4753935	173	$ 830.00	8717	06/28/88	01/30/76	25900
4756828	173	$ 830.00	599386	07/12/88	04/12/84	92100
4757004	173	$ 830.00	591155	07/12/88	03/16/84	92110
4758587	173	$ 830.00	23590	07/19/88	03/mr/87	26030
4760030	173	$ 830.00	649253	07/26/88	09/10/84	24530
4770874	173	$ 830.00	859665	09/13/88	05/05/86	24000 DIV 1
4772466	173	$ 830.00	703791	09/20/88	02/21/85	24000 CIP 2
4772697	173	$ 830.00	898559	09/20/88	08/21/86	92030 DIV 1
4774191	173	$ 830.00	826177	09/27/88	02/05/86	20940 DIV 3

Please charge any additional fees for the maintenance of these cases at this time or credit any overpayment to Deposit Account No. 19-5430.

Respectfully submitted,

DAL LH15189 11/14/91 4753935 19-5430 130 173 830.00CH

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Date Mailed: October 31, 1991

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I hereby certify that this correspondence and patent application are being deposited with the United States Postal Service as "EXPRESS MAIL - POST OFFICE TO ADDRESS-EE" under 37 CFR 1.10 in an envelope addressed to the Commissioner, Patents and Trademarks, Washington, D.C. 20231,

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